what circumstances are associated with proteasome-dependent degradation of FOXP3. Whether the findings of the present papers speak more to the prevention of maximal induction of FOXP3 or the effective loss of FOXP3 in fully differentiated nTreg cells remains to be established.

Regardless, the implication that inflammatory cytokines can strip FOXP3 from a Treg cell does not simply provide more fuel for an academic fire; what makes this work of real clinical importance is the possibility of manipulating FOXP3 and Treg cells in autoimmunity and transplantation. Conversely, the therapeutic utility of attenuating regulatory immune mechanisms is one of the most exciting developments in cancer immunology. Both groups were mindful in demonstrating that ubiquitination is equally as important in human and murine Treg cells. Proteasome inhibitors are already used in the treatment of myeloma and mantle cell lymphoma, and it may not be long before they are used in the regulation of autoimmune disease.

REFERENCES

Abbas, A.K., Benoist, C., Bluestone, J.A., Campbell, D.J., Ghosh, S., Hori, S., Jiang, S., Kuchroo, V.K., Mathis, D., Roncarolo, M.G., et al. (2013). Nat. Immunol. *14*, 307–308.

Chen, Z., Barbi, J., Bu, S., Yang, H.-Y., Li, Z., Gao, Y., Jinasena, D., Fu, J., Lin, F., Chen, C., et al. (2013). Immunity *39*, this issue, 272–285.

Dang, E.V., Barbi, J., Yang, H.Y., Jinasena, D., Yu, H., Zheng, Y., Bordman, Z., Fu, J., Kim, Y., Yen, H.R., et al. (2011). Cell *146*, 772–784.

Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., Schlawe, K., Chang, H.D., Bopp, T., Schmitt, E., et al. (2007). PLoS Biol. *5*, e38.

Miyao, T., Floess, S., Setoguchi, R., Luche, H., Fehling, H.J., Waldmann, H., Huehn, J., and Hori, S. (2012). Immunity *36*, 262–275.

Oldenhove, G., Bouladoux, N., Wohlfert, E.A., Hall, J.A., Chou, D., Dos Santos, L., O'Brien, S., Blank, R., Lamb, E., Natarajan, S., et al. (2009). Immunity *31*, 772–786.

Rubtsov, Y.P., Niec, R.E., Josefowicz, S., Li, L., Darce, J., Mathis, D., Benoist, C., and Rudensky, A.Y. (2010). Science *329*, 1667–1671.

Sakaguchi, S., Vignali, D.A., Rudensky, A.Y., Niec, R.E., and Waldmann, H. (2013). Nat. Rev. Immunol. *13*, 461–467.

van Loosdregt, J., Fleskens, V., Fu, J., Brenkman, A.B., Bekker, C.P.J., Pals, C.E.G.M., Meerding, J., Berkers, C.R., Barbi, J., Gröne, A., et al. (2013). Immunity *39*, this issue, 259–271.

Weiss, J.M., Bilate, A.M., Gobert, M., Ding, Y., Curotto de Lafaille, M.A., Parkhurst, C.N., Xiong, H., Dolpady, J., Frey, A.B., Ruocco, M.G., et al. (2012). J. Exp. Med. *209*, 1723–1742, S1.

Zhou, X., Bailey-Bucktrout, S.L., Jeker, L.T., Penaranda, C., Martínez-Llordella, M., Ashby, M., Nakayama, M., Rosenthal, W., and Bluestone, J.A. (2009). Nat. Immunol. *10*, 1000–1007.

Generating CD8 T Cell Heterogeneity: Attack of the Clones

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Pathogen-induced inflammation modulates CD8 T cell effector and memory differentiation. In this issue of *Immunity*, Plumlee et al. (2013) demonstrate that clonally distinct CD8 T cells have the ability to generate numerous types of effector cell fates based on extrinsic pathogen-induced environmental cues.

During infection, individual naive pathogen-specific T cells receive signals that incite exponential growth and effector differentiation in order to rid the body of the pathogen. After pathogen clearance, most of the effector T cells undergo apoptosis, but a small proportion of cells survive to differentiate into mature memory T cells that, together with long-lived plasma cells and memory B cells, provide protection upon reinfection. As effector CD8 T cells expand and differentiate, they give rise to numerous phenotypically, functionally, and anatomically distinct subsets, which in turn give rise to diverse pools of memory CD8 T cells. Some effector cell subsets are inherently more fit to persist long-term and populate the memory cell pool, and in many cases these cells can be identified based on increased expression of interleukin-7Rα (IL-7Rα, CD127), CD27, and B cell lymphoma 2 (Bcl2) (Kaech and Cui, 2012). Understanding the basis of diversity in effector CD8 T cell function, migration, and memory cell potential might help inform the generation of more efficacious vaccines against pathogens and cancers. In the current issue of *Immunity*, Plumlee et al. (2013) establish that extrinsic pathogen-induced environmental cues shape the differentiation of individual naive CD8 T cell clones during infection.

T cell effector and memory differentiation is influenced by the type, timing, strength, and duration of antigenic (signal 1), costimulatory (signal 2), and cytokine (signal 3) signaling. Different infections modulate these signals by infecting

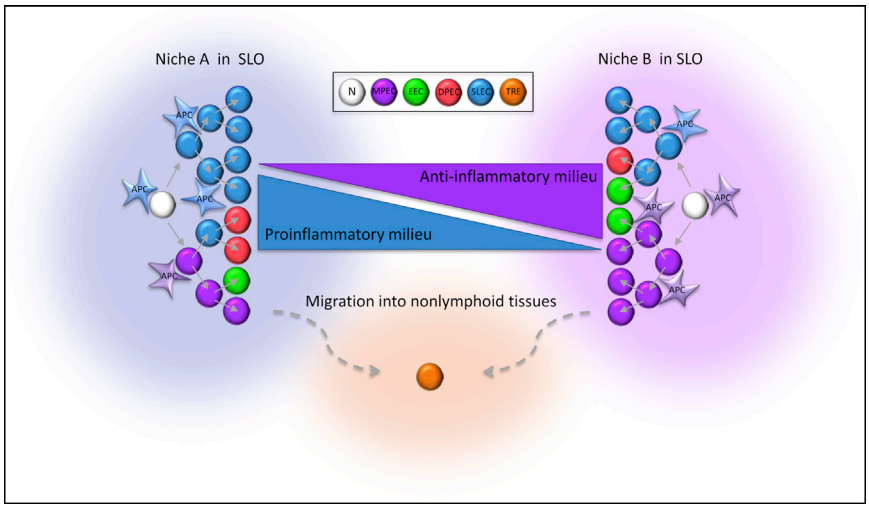

Figure 1. Distinct Priming Niches Modulate CD8 T Cell-Fate Decisions during Infection
This model postulates that in SLOs, the microenvironment of APC-T cell interactions can vary with respect to the presence and abundance of inflammatory cytokines. Niches, as exemplified by Niche A (blue), with a higher density of proinflammatory cells and APCs and reduced frequency of anti-inflammatory or regulatory cells will promote the expansion and development of short-lived effector cell (SLEC) and double-positive effector cell (DPEC) phenotypes in CD8 T cell clones, whereas less inflammatory niches, as exemplified by Niche B (purple), will permit a greater fraction of effector cells to adopt memory precursor effector cell (MPEC) and early effector cell (EEC)-like states. Following migration to nonlymphoid tissues, the signaling milieu at these sites can also impart distinct cell fates for tissue-resident effectors cells (TRE).

distinct tissues, regulating antigen presentation and abundance, and inducing diverse milieus of pro- and anti-inflammatory cytokines. Numerous studies have utilized genetic tools to modulate individual signaling pathways in both polyclonal and monoclonal T cell receptor (TCR) transgenic (Tg) T cell populations during infection. For example, several studies highlight the propensity of proinflammatory signals, such as IL-2, IL-12, and IL-27, to promote terminal effector (TE) differentiation and anti-inflammatory signals, such as IL-10, to preserve memory potential in a subset of CD8 T cells (referred to as memory precursor [MP] cells; references within Kaech and Cui, 2012) (Figure 1). Other work demonstrates that the abundance of antigen and differences in TCR affinity for pMHC can impact effector T cell expansion and differentiation, in part by altering the dwell time of T cells with antigen-presenting cells (APCs) and asymmetric partitioning of cell-fate determinants during cell division (Chang et al., 2007; King et al., 2012; Zehn et al., 2009). Together, this work indicates that the type, strength, timing, and duration of these various signaling pathways within even monoclonal T cells can differ, resulting in diverse outputs on effector cells. Therefore, understanding how individual naive T cell clones differentiate into effector and memory cells will help to distinguish important signals received during priming versus those received by daughter cells later in the response.

In the elegant study by Plumlee et al. (2013), a single cell transfer system was established to track the fate of individual polyclonal CD8 T cell precursors. Based on the known precursor frequency of OVA-specific CD8 T cells in C57BL/6 mice, the authors adoptively transferred ~1 OVA-specific naive CD8 T cell per mouse, infected mice with VSV-OVA or *Listeria*-OVA, and analyzed the phenotype of the effector CD8 T cell clones that formed. The populations of effector CD8 T cells were verified as clonal by TCR sequencing and the size and the phenotype of the responding clonal populations after the two infections was interrogated based on varying expression of CD127 and killer cell lectin-like receptor G1 (KLRG1). For simplicity, the different subsets of effector CD8 T cells were referred to as short-lived effector cells (SLEC, CD127^{lo} KLRG1^{hi}), double-positive effector cells (DPEC, CD127^{hi} KLRG1^{hi}), early effector cells (EEC, CD127^{lo} KLRG1^{lo}), and memory precursor effector cells (MPEC, CD127^{hi} KLRG1^{lo}). The microenvironment in which the CD8 T cell clone was generated had a profound influence on the types of daughter cells produced. For example, during VSV infection, a larger fraction of the effector CD8 T cell clones displayed MPEC and EEC phenotypes, whereas during *Listeria* infection, the majority of the cell clones acquired SLEC phenotypes. This indicated that the distinct cytokine milieu induced by these different infections and other extrinsic signals, possibly antigen presentation or abundance, modulates effector CD8 T cell differentiation at the clonal level. During oral *Listeria*-OVA infection in a single mouse, clonal CD8 T cells residing in the spleen were more heavily biased toward SLEC phenotype, whereas progeny of the same clone in the gut were more MPEC/EEC biased. Thus, in addition to pathogen-induced environmental fluctuations encountered during T cell priming, tissue-derived environmental factors also contribute to the generation of diverse types of effector CD8 T cells during infection (Figure 1). Lastly, the authors also demonstrate that clonal memory CD8 T cells are inherently biased to differentiate into KLRG1^{hi} secondary effectors, even during VSV infection, which induces more KLRG1^{lo} phenotype primary effector cells. These data suggest that the differentiation of secondary effectors from resting memory cells are more heavily influenced by intrinsic factors, such as epigenetic gene-regulatory changes or their propensity to rapidly upregulate T-bet (Joshi et al., 2011), than extrinsic cues. This further highlights the need to dissect the contribution of intrinsic and extrinsic signals on individual naive and memory T cell clones to understand the contribution of all of these signals to the overall immune response and memory T cell formation.

Other recent, elegant studies have tracked the fate of single naive CD8 T cells via single T cell transfers or barcoding of TCR Tg CD8 T cells (Buchholz et al., 2013; Gerlach et al., 2013; Lemaître et al., 2013). However, these studies differ in a few important ways. One is that those prior examined the clonal progeny of TCR Tg naive CD8 T cells, whereas Plumlee et al. (2013) studied the descendants of polyclonal naive T cell precursors. The latter would allow for differences in TCR affinity to

have a greater influence on effector cell differentiation. Additionally, the prior studies only examined the heterogeneity of effector CD8 T cells during a single type of infection, whereas the present study encompasses two infections and multiple tissues to compare the effects of different infectious environments on the differentiation of individual effector CD8 T cell clones. Additional key points that arise collectively from these studies are that the progeny of individual naive T cells are plastic and have the ability to generate heterogeneous effector and memory T cell populations. However, each clone is rather unique in the proportion of cells that adopt particular fates. Conceptually similar findings were provided by studies analyzing the generation of T helper 1 (Th1) and T follicular helper (Tfh) effector cells from single naive CD4 T cell precursors (Tubo et al., 2013). These data clearly illustrate that the bulk polyclonal effector T cell response for any given infection is actually an average of the variation in phenotypes of the individual clones. In addition, there is marked variability in the size of each clonal response, which often correlates with their phenotype (e.g., the largest clones were enriched with $CD62L^{lo}$ $KLRG1^{hi}$ cells) (Plumlee et al., 2013; Buchholz et al., 2013; Gerlach et al., 2013). This suggests that the differentiation state of T cells is linked to the number of times the cell divided, but it is not known whether the variation in clone size and phenotype is attributed to the number of times antigen is encountered and/or the types of APCs encountered. Further, increasing TCR-pMHC dwell time can also influence the types of effector cells formed, promoting the development of $CD4^+$ Tfh or $CD8^+$ TE cells, respectively (King et al., 2012; Tubo et al., 2013). It will be important to more closely dissect how the strength of TCR and inflammatory cytokine signaling are integrated during effector CD8 T cell differentiation.

In conclusion, CD8 T cells must integrate many distinct signals from the microenvironment in secondary lymphoid organs (SLO) or infected tissues, and it will remain important to dissect out the contribution of these different signaling pathways on the generation of effector and memory T cells in order to make more efficacious vaccines. Importantly, as a result of the high clonal variance in expansion and the correlation with phenotype, understanding how TCR affinity and/or avidity shapes the differentiation of individual T cell clones will be a necessary first step. It will also be important to assess when daughter cells from individual clones adopt particular differentiation states and whether the quality of the signals first encountered by the earliest progenitors are ''imprinted'' into the clone or whether the daughter cells remain fairly plastic for several days and gradually adopt distinct phenotypes based on the collection of signals experienced. A better understanding of how distinct niches in SLO and peripheral tissues form during infection and influence the formation and function of effector and memory CD8 T cells that reside within these niches will need to be addressed. Finally, assessment of the epigenetic landscape within clonal T cell populations could help explain when, where, and for whom T cell fates are fixed.

REFERENCES

Buchholz, V.R., Flossdorf, M., Hensel, I., Kretschmer, L., Weissbrich, B., Gräf, P., Verschoor, A., Schiemann, M., Höfer, T., and Busch, D.H. (2013). Science *340*, 630–635.

Chang, J.T., Palanivel, V.R., Kinjyo, I., Schambach, F., Intlekofer, A.M., Banerjee, A., Longworth, S.A., Vinup, K.E., Mrass, P., Oliaro, J., et al. (2007). Science *315*, 1687–1691.

Gerlach, C., Rohr, J.C., Perié, L., van Rooij, N., van Heijst, J.W., Velds, A., Urbanus, J., Naik, S.H., Jacobs, H., Beltman, J.B., et al. (2013). Science *340*, 635–639.

Joshi, N.S., Cui, W., Dominguez, C.X., Chen, J.H., Hand, T.W., and Kaech, S.M. (2011). J. Immunol. *187*, 4068–4076.

Kaech, S.M., and Cui, W. (2012). Nat. Rev. Immunol. *12*, 749–761.

King, C.G., Koehli, S., Hausmann, B., Schmaler, M., Zehn, D., and Palmer, E. (2012). Immunity *37*, 709–720.

Lemaître, F., Moreau, H.D., Vedele, L., and Bousso, P. (2013). J. Immunol. Published online July 8, 2013. http://dx.doi.org/10.4049/jimmunol.1300424.

Plumlee, C.R., Sheridan, B.S., Cicek, B.B., and Lefrançois, L. (2013). Immunity *39*, this issue, 347–356.

Tubo, N.J., Pagán, A.J., Taylor, J.J., Nelson, R.W., Linehan, J.L., Ertelt, J.M., Huseby, E.S., Way, S.S., and Jenkins, M.K. (2013). Cell *153*, 785–796.

Zehn, D., Lee, S.Y., and Bevan, M.J. (2009). Nature *458*, 211–214.